UNIVERSITI PUTRA MALAYSIA

Andrographis paniculata Nees AND Orthosiphon stamineus Benth
GROUND LEAF AS ANTIBIOTIC AND ANTIOXIDANT SUPPLEMENTS
FOR BROILER CHICKEN

MASNINDAH BINTI MALAHUBBAN

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

September 2014
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GROUND LEAF AS ANTIBIOTIC AND ANTIOXIDANT SUPPLEMENTS FOR BROILER CHICKEN

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MASNINDAH BINTI MALAHUBBAN

September 2014

Chairman: Professor Abd. Razak Bin Alimon, Ph.D.

Faculty: Institute of Tropical Agriculture

The use of synthetic antibiotics and antioxidants has created serious obstacles and hazards to farmers, consumers and the environment. Antibiotics and antioxidant from natural sources can potentially be useful to overcome these problems. Therefore, present study was conducted to evaluate the selected medicinal plants, Andrographis paniculata and Orthosiphon stamineus for antibiotics and antioxidant potential and subsequently to evaluate the broiler performance by inclusion the A. paniculata and O. stamineus ground leaf in diets.

Initially, A. paniculata and O. stamineus ground leaf were evaluated for their in vitro antibiotic and antioxidant potential by extracting the ground leaf in four solvents, namely water, methanol, ethanol and chloroform and subsequently testing using the disc diffusion method for antibiotic potential and selected radical scavenging activities for antioxidant potential. Qualitative screening for bioactive compound on both extracts by using methanol as solvent has indicated the presence of alkaloid, saponin, flavonoid, tannin, terpenoid and steroid. High performance liquid chromatography analysis indicated that andrographolide and rosmarinic acid were the major compounds from A. paniculata and O. stamineus, respectively. It was found that the highest yields of these two compounds were obtained by methanol extraction and that substantial antibiotic and antioxidant properties were exhibited by these compounds. In addition, it was showed that the O. stamineus extract contained higher antioxidant capacity than A. paniculata extract.
The ground leaf were incorporated in broiler diets at levels of 0, 2, 4, and 8 g/kg and the influence on growth performance, carcass characteristics, serum biochemistry, and intestinal and liver morphology was examined in a 42-d feeding trial. It was involved 280 one-day old male broiler chickens, grown, maintained and received ad libitum water and diet. The feeding trial was started from 21 days old and assigned with respective treatments. At day-42, the broiler chickens were slaughtered and analysed and it was found that broilers fed O. stamineus ground leaf at a rate 8 g/kg was the most promising dietary supplement to promote overall growth performance without deleterious effects on carcass characteristics, serum biochemical properties and morphological components of liver and intestine compared with A. paniculata ground leaf and control diets. In addition to promoting weight gain, it reduced abdominal fat and serum cholesterol. It also maintained the integrity of liver, thus indicating that no toxic effect from O. stamineus supplementation at a rate up to 0.8 g/kg. Besides that, 8 g/kg O. stamineus supplementation improved intestinal structure, especially in the duodenum. Present study also found that the inclusion of O. stamineus ground leaf at 8 g/kg in the broiler diet increased total tract N retention and apparent metabolizable energy.

Based on these results, O. stamineus ground leaf at 8 g/kg was selected for the next in vivo experiment. Present study was conducted to evaluate the response of broiler due to antibiotic and antioxidant properties of O. stamineus by comparing its potential with tetracycline and Vitamin E supplementation in diets, as positive controls. The study was involved 160 of a one-day old male broiler chickens, and had ad libitum water and feed for up to 20-day old. The respective treatments were assigned and initiated at 21-day old male broiler chickens. After slaughtering, data were recorded and analysed at 42-day old male broiler chickens, and it was found that O. stamineus ground leaf supplement at a rate of 8 g/kg in broiler diet results in growth performance similar to that of tetracycline and Vitamin E supplementation. In addition, it was also found that 8 g/kg O. stamineus supplementation in diet promoted serum enzymes-lowering effect. In contrast, high serum enzymes activity showed in broiler fed tetracycline supplement. The inclusion of 8 g/kg O. stamineus ground leaf in diet of broiler enhanced meat quality by stabilizing sensory properties, meat colour and meat pH. The results also indicated that the inclusion of O. stamineus leaf ground at 8 g/kg in diet was comparable with 200 mg/kg Vitamin E supplementation in diet. It was found that all dietary treatments maintained intestinal population of Lactobacillus and Escherichia coli. However, the inclusion of 8 g/kg O. stamineus or tetracycline in diet inhibited the population of facultative anaerobe. Therefore, the use of O. stamineus ground leaf as supplement in broiler chicken diet has the potential to promote and maintain growth and gut health and subsequently creates safe and sustainable broiler chicken production.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

**SERBUK DAUN** *Andrographis paniculata* **Nees** **DAN** *Orthosiphon stamineus* **Benth** **SEBAGAI ADITIF ANTIBIOTIK DAN ANTIOKSIDAN UNTUK AYAM PEDAGING

Oleh

MASNINDAH BINTI MALAHUBAN

September 2014

Pengerusi: Profesor Abd. Razak Bin Alimon, Ph.D.

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Penggunaan antibiotik dan antioksidan sintetik telah menyebabkan masalah dan bencana yang serius kepada petani, pengguna dan persekitaran. Antibiotik dan antioksidan dari sumber semulajadi dilihat berpotensi untuk digunakan bagi mengatasi masalah ini. Maka kajian ini dijalankan untuk menilai tumbuhan perubatan yang terpilih iaitu *Andrographis paniculata* dan *Orthosiphon stamineus* yang berpotensi sebagai sumber antibiotik dan antioksidan, seterusnya kajian ini turut menilai prestasi ayam pedaging kesan dari pengambilan makanan yang mengandungi serbuk daun *A. paniculata* dan *O. stamineus*.

Kajian dimulakan dengan menilai potensi antibiotik dan antioksidan serbuk daun *A. paniculata* dan *O. stamineus* secara in vitro dengan mengekstrak serbuk daun tersebut dalam empat jenis bahan pelarut iaitu air, methanol, etanol dan klorofom, dan seterusnya ia diuji menggunakan kaedah sebaran cakera bagi menentukan potensi antibiotik dan aktiviti-aktiviti pencarian radikal bagi penentuan potensi antioksidan. Saringan kualitatif telah dijalankan, dan ia menunjukkan kedua-dua serbuk daun yang diekstrak menggunakan metanol mengandungi alkaloid, saponin, flavonoid, tannin, terpenoid dan steroid. Analisis kromatografi cecair berprestasi tinggi menunjukkan andrographolida dan asid rosmarinik merupakan kompaun utama masing-masing dari *A. paniculata* dan *O. stamineus*. Kajian mendapati hasilan paling tinggi bagi kedua-dua kompaun terbabit diperoleh daripada pengekstrakan methanol dan ciri-ciri antibiotik dan antioksidan yang menggalakkan ditunjukkan oleh kedua-dua kompaun terbabit.

Serbuk daun berkenaan dicampurkan ke dalam diet ayam pedaging pada beberapa paras kandungan iaitu 0, 2, 4, dan 8 g/kg bagi menentukan pengaruhnya ke atas prestasi pertumbuhan, ciri-ciri karkas, biokimia serum,
dan morfologi usus dan hati, dan kajian cubaan pemakanan ini dilakukan sehingga ayam pedaging berusia 42 hari. Kajian ini melibatkan ayam pedaging jantan yang diperoleh pada ketika usianya satu hari, dipelihara dan dijaga. Cubaan pemakanan dimulakan ketika ayam pedaging berusia 21 hari dan seterusnya disusun mengikut rawatan tersebut. Pada usianya 42 hari, ayam pedaging disembelih dan dianalisis, dan keputusan mendapati ayam pedaging yang makan 8 g/kg serbuk daun *O. stamineus* merupakan makanan tambahan yang paling berpotensi untuk merangsang prestasi pertumbuhan tanpa kesan-kesan negative ke atas ciri-ciri karkas, kandungan biokimia serum dan komponen morfologi hati dan usus berbanding dengan serbuk daun *A. paniculata* dan diet-diet kawalan. Tambahan kepada penggalakan peningkatan berat, ia telah mengurangkan lemak perut dan kolesterol serum. Ia juga memelihara integriti hati, dan hal ini menunjukkan tiadanya kesan toksik dari *O. stamineus* yang ditambah di dalam pemakanan sebanyak 8 g/kg. Di samping itu, penambahan 8 g/kg *O. stamineus* memperbaiki struktur usus, terutamanya duodenum. Kajian ini turut mendapati penambahan 8 g/kg serbuk daun *O. stamineus* meningkatkan jumlah zon pengumpulan N dan tenaga sebenar yang boleh dimetabolikkan.

Berdasarkan kepada keputusan tersebut, 8 g/kg serbuk daun *O. stamineus* telah dipilih untuk eksperimen *in vivo*. Kajian ini dijalankan untuk menilai tindakbalas ayam pedaging kesan dari kandungan antibiotik dan antioksidan *O. stamineus* dengan membandingkannya dengan penambahan tetrasiiklin dan Vitamin E di dalam pemakanan, sebagai kawalan positif. Kajian ini melibatkan 160 ekor ayam pedaging jantan yang dipelihara sejak berusia satu hari. Rawatan kajian dimulakan semasa ayam pedaging berusia 21 hari. Data mula direkodkan dan dianalisis ketika berusia 42 hari. Kajian menunjukkan serbuk daun *O. stamineus* pada kadar 8 g/kg mempunyai prestasi pertumbuhan yang menyamai prestasi tetrasiiklin dan Vitamin E. Tambahan lagi, ia juga didapati mampu merangsang kesan kekurangan enzim serum. Sebaliknya, aktiviti enzim serum yang tinggi didapati pada ayam pedaging yang dirawat dengan pemakanan tambahan yang mengandungi tetrasiiklin. Penambahan 8 g/kg serbuk daun *O. stamineus* di dalam makanan ayam pedaging telah meningkatkan kualiti daging dengan menstabilkan komponen rasa, warna dan pH daging. Kajian turut menunjukkan ia setanding dengan penambahan sebanyak 200 mg/kg Vitamin E. Selain itu, rawatan tambahan pemakanan ini memelihara populasi *Lactobacillus* dan *Eschericia coli* di dalam usus. Walau bagaimanapun, penambahan 8 g/kg serbuk daun *O. stamineus* merencanakan populasi fakultatif anaerob. Dengan ini, penggunaan serbuk daun *O. stamineus* sebagai tambahan di dalam pemakanan ayam pedaging berpotensi untuk merangsang dan mekekalkan pertumbuhan dan kesihatan perut ayam pedaging dan seterusnya mewujudkan pengeluaran ayam pedaging yang selamat dan lestari.
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I certify that a Thesis Examination Committee has met on (date of viva) to conduct the final examination of Masnindah Binti Malahubban on her thesis entitled “Andrographis paniculata Nees AND Orthosiphon stamineus Benth Ground Leaf as Antibiotic and Antioxidant Supplements for Broiler Chicken” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the students be awarded the Doctor of Philosophy.

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5.1 The pH of raw breast meat of broiler chickens on different diets. Changes over duration of storage at 4°C.
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<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>AME</td>
<td>Apparent metabolizable energy</td>
</tr>
<tr>
<td>AP</td>
<td>Andrographis paniculata</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Aminotransferase</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Unit</td>
</tr>
<tr>
<td>CP</td>
<td>Crude Protein</td>
</tr>
<tr>
<td>DM</td>
<td>Dry Matter</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<tr>
<td>FCR</td>
<td>Food Conversion Ratio</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Association</td>
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<tr>
<td>GE</td>
<td>Gross Energy</td>
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<tr>
<td>GAE</td>
<td>Gallic Acid Equivalent</td>
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<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Inhibitory Concentration at 50 %</td>
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<tr>
<td>NRC</td>
<td>National Research Council</td>
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<tr>
<td>OS</td>
<td>Orthosiphon stamineus</td>
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<tr>
<td>T20</td>
<td>Tetracycline</td>
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<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
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<tr>
<td>VE</td>
<td>Vitamin E</td>
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<tr>
<td>Units</td>
<td>Description</td>
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<td>-------</td>
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<tr>
<td>°C</td>
<td>degrees centigrade</td>
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<tr>
<td>cm</td>
<td>centimeter</td>
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<tr>
<td>g</td>
<td>gram</td>
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<tr>
<td>g/day</td>
<td>gram per day</td>
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<tr>
<td>g/kg</td>
<td>gram per kilogram</td>
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<tr>
<td>h</td>
<td>hour</td>
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<tr>
<td>l</td>
<td>liter</td>
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<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>MJ/kg</td>
<td>megajoule per kilogram</td>
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<tr>
<td>min</td>
<td>minute</td>
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<tr>
<td>mbar</td>
<td>milibar</td>
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<tr>
<td>mg</td>
<td>milligram</td>
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<tr>
<td>ml</td>
<td>milliliter</td>
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<td>mm</td>
<td>millimeter</td>
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<tr>
<td>mmol/L</td>
<td>milimol per liter</td>
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<td>ml/min</td>
<td>milliliter per minute</td>
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<td>mM</td>
<td>milimol</td>
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<tr>
<td>mg/ml</td>
<td>milligram per milliliter</td>
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<tr>
<td>M</td>
<td>molar</td>
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<tr>
<td>µm</td>
<td>micrometer</td>
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<td>µL</td>
<td>microliter</td>
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<td>µg</td>
<td>microgram</td>
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<td>ppm</td>
<td>parts per million</td>
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<td>sec</td>
<td>second</td>
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Common abbreviations

et al. and others
%
percentage
e.g. for example

Statistical terms

ANOVA Analysis of variance
CRD Completely Randomized Design
SE Standard Error

Chemical elements and compounds

ABTS 2,2- azinobis- (3 ethylbenzothiazoline-6-sulfonic acid)
ACN acetonitrile
DPPH 2, 2- diphenyl -1-picrylhydrazyl
HCl Hydrochloric acid
H₂SO₄ Sulphuric acid
MHA Muller Hinton Agar
RA Rogosa Agar
CHAPTER 1

GENERAL INTRODUCTION

Broiler chicken represents 29% of meat production from farmed animals and this value rising each year. Poultry meat and eggs offer considerable potential for meeting human needs for dietary animal protein supply. With regards to the growth of broiler industry, Malaysia ranked seventh place in the world (Anon, 2012). In Malaysia, the broiler industry is expected to grow at the rate of 4% in 2013, outpacing global production growth at 2.5% (Lim, 2013).

To improve broiler production and satisfy market demand, it has been the common practice for farmers to use synthetic antibiotics and antioxidant in the feed as growth promoters. Antibiotics including chlortetracycline, tetracycline, virginiamycin, spiramycin, tylosin phosphate, zinc bacitracin and avopacrin as growth promoters have been used for decades in poultry production for improving farm performance and controlling diseases (Huyghebaert et al., 2011). With increasing interests in discontinuing the use of antibiotics due to their harmful effects to environment, actively efforts to search for safe, suitable and viable alternatives to the antibiotic growth promoters has become intensified. Ideally, these alternative growth promoters should improve growth performance, as do the antibiotics, and maintain a sound health of the chickens. Besides the need of healthy grown broilers, meat quality also needs to give much attention. Oxidative stability is a central parameter in the estimation of meat quality because of the susceptibility of this food product to oxidative degeneration, which is one of the main causes of spoilage (Morrissey et al., 1998). The shelf life of meat is related to lipid oxidation reactions which could affect its sensory properties, by causing rancidity, as well as its nutritional characteristics through the formation of potentially toxic compound (Morrissey and Kerry, 2004). Conventionally, oxidation of chicken meat can be prevented by synthetic antioxidants including butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG) but their safety has been questioned (Barlow, 1990).

Moreover, the excessive use of these synthetic antibiotics and antioxidants has led to contamination of broiler meat and environment. For example, the practice of feeding antibiotics to livestock leads to antibiotic-resistant bacteria that are dangerous to human health. Frequently and excessively used of antibiotics cause harmful bacteria become resistant to the drug, and the treatment becomes less effective (Huyghebaert et al., 2011). In fact, bacteria can develop into a completely different strain that cannot be killed by the normally prescribed antibiotic. Synthetic antioxidants have been restricted recently, mainly because of their possible carcinogenicity causing liver swelling and changing liver enzyme activities. With the increasing consciousness of public consumers on safety of food additive, therefore, an urgent need for identifying alternative natural and probably safer sources of antibiotics and antioxidants.
Currently, the interest in natural antioxidants and antibiotics have increased dramatically because they are considered to be safer than the synthetics, and have greater application potential for consumers acceptability, palatability, stability and shelf-life of meat products (Kang et al., 2008). The medicinal herbs have been used since ancient times not only for flavouring foods but also for their remedies. The preservative effect of spices and herbs suggests the presence of antimicrobial and antioxidative constituents (Basmacioglu et al., 2004; Al-Marzooqi et al., 2010).

In the present study, two species of medicinal plants have been selected namely, Andrographis paniculata Nees, (Acanthaceae) and Orthosiphon stamineus Benth, (Lamiaceae), they are common in Southeast Asia, India and China. A. paniculata has been traditionally used as an antioxidant, antiviral, anti-inflammatory, immune enhancing agent and hepatoprotective (Prajjal et al., 2003). Its active component has been reported to have anti-cancer (Sheeja and Kuttan, 2007), anti-HIV (Calabrese et al., 2000), and antimicrobial (Roy et al., 2010) properties. The other herb, Orthosiphon stamineus, has been used to treat urinary lithiasis, edema, eruptive fever, influenza, rheumatism, hepatitis, jaundice and biliary lithiasis (Akowuah et al., 2005). O. stamineus leaf is consumed as Java tea to facilitate body detoxification (Chin et al., 2008). Both plants have been reported containing natural antibiotics and antioxidants (Prajjal et al., 2003; Ho et al., 2010). However, very few information of medicinal plants as dietary supplements in animals, especially Andrographis paniculata and Orthosiphon stamineus. The hypotheses of the present study were:

1. Andrographis paniculata and Orthosiphon stamineus are species of medicinal plants containing antibiotic and antioxidant compounds.

The goal of this research work was to evaluate the possibilities of improving broiler chicken performance using A. paniculata and O. stamineus leaf preparation as feed supplement as opposed to the synthetic antibiotics and antioxidant. The specific objectives set were:

1. To determine the antibiotic and antioxidant properties of A. paniculata and O. stamineus leaf extracts in different solvents, and to detect and quantify andrographolide and rosmarinic acid presence in the respective species.
2. To evaluate the effect of different levels of A. paniculata and O. stamineus ground leaf supplemented in diets on growth performance, carcass characteristics and blood biochemistry of broiler chickens, and also to determine the morphological and histological changes in gastrointestinal tract and in the liver.
3. To evaluate the antibiotic and antioxidant potential of Orthosiphon stamineus ground leaf on growth performance, meat characteristics, intestinal microflora and lipid peroxidation of broiler chickens.


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